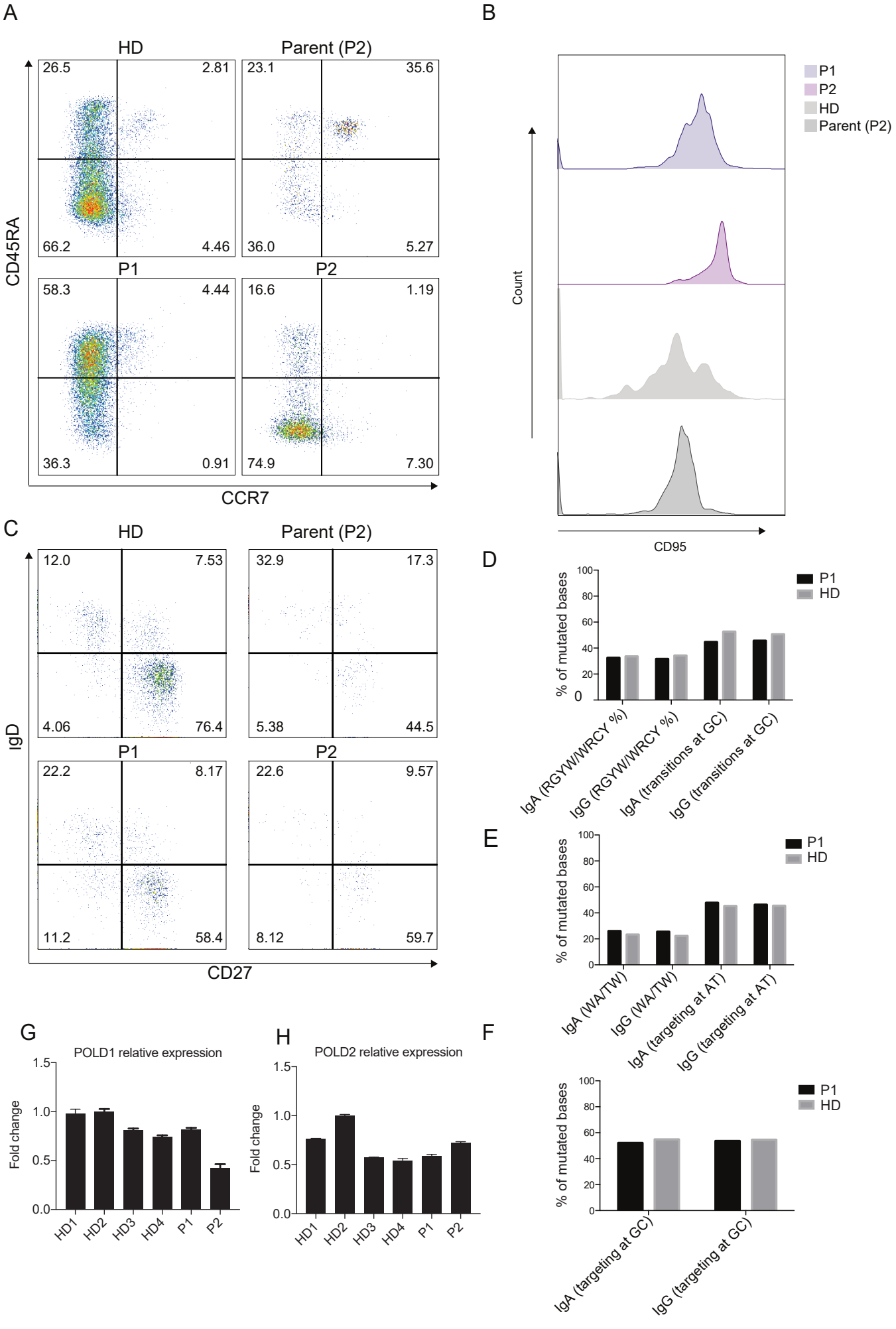
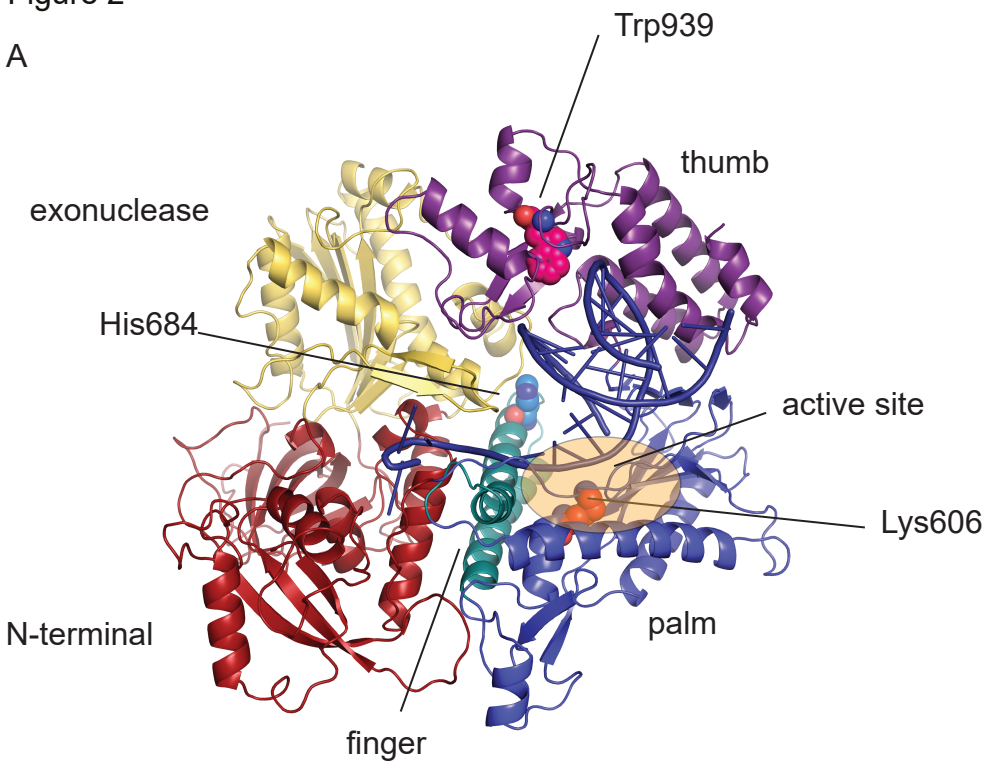
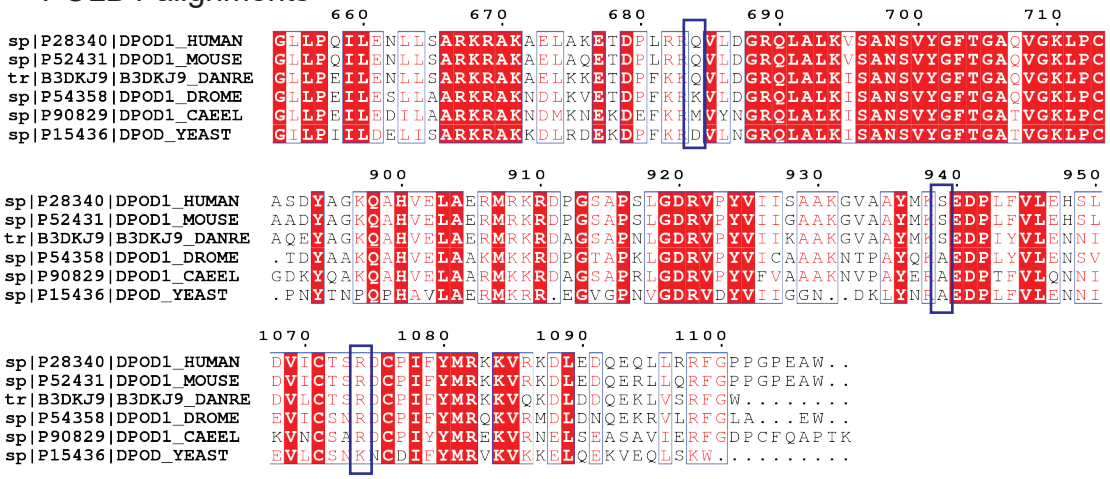
Supplementary Figure 1



Supplementary Figure 2

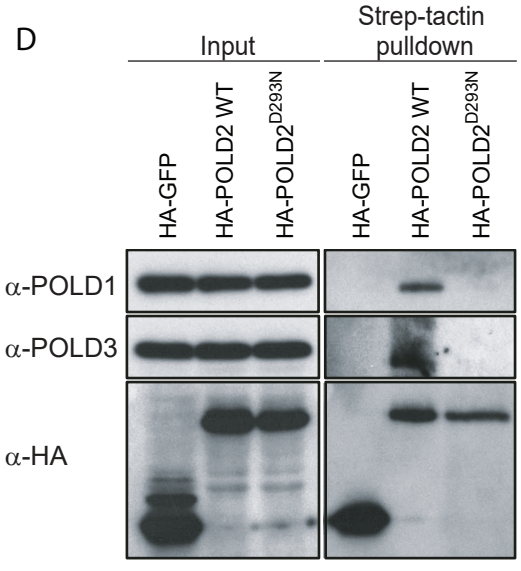


B POLD1 alignments



C POLD2 alignment

D



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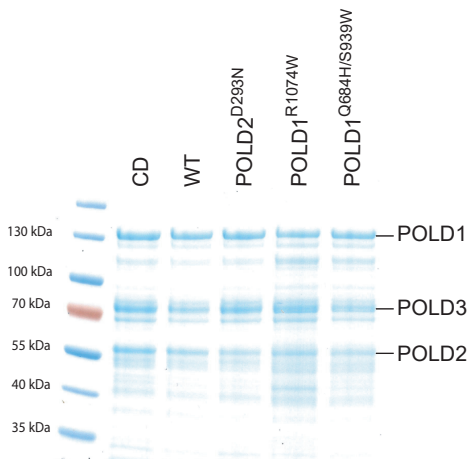
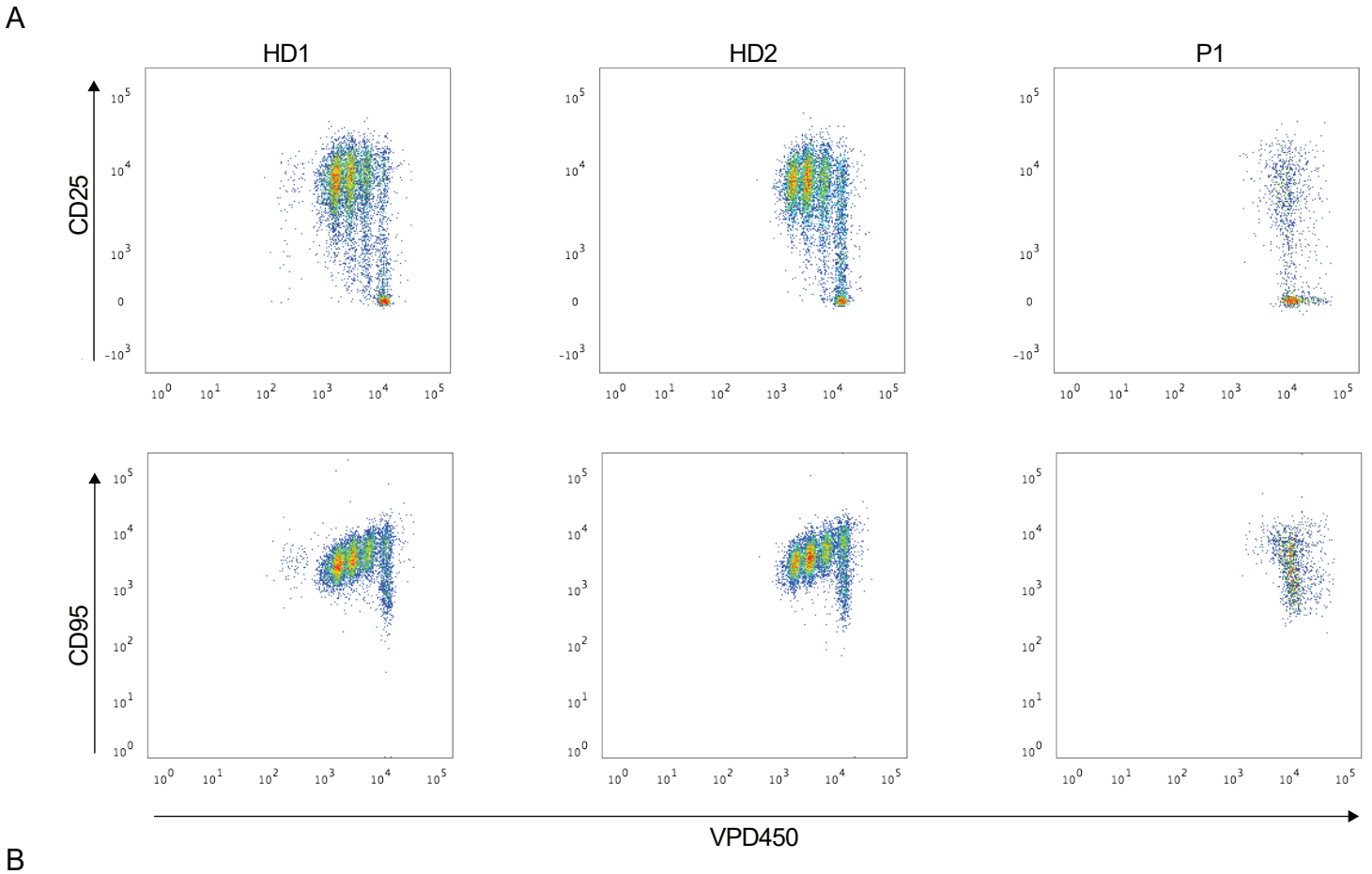
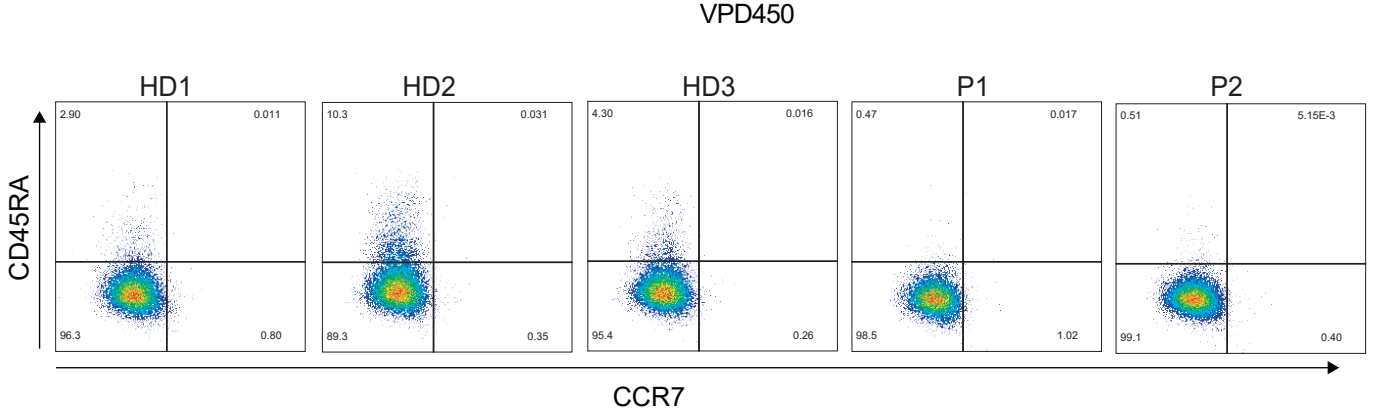
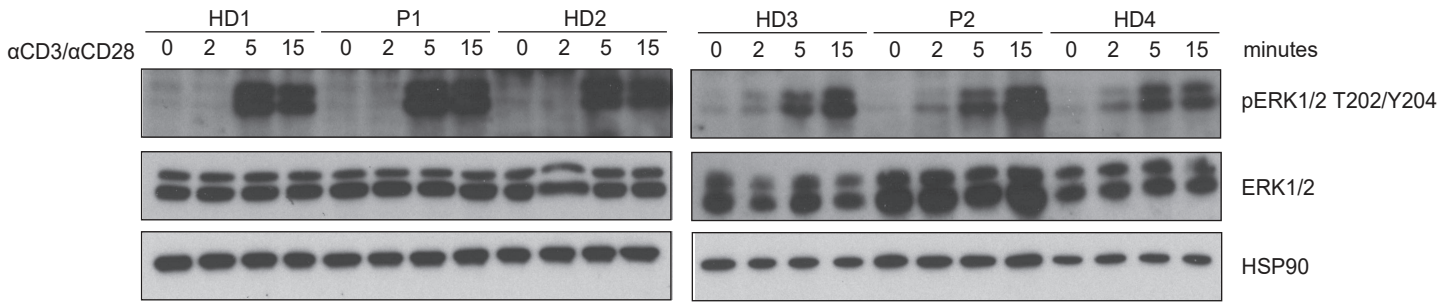


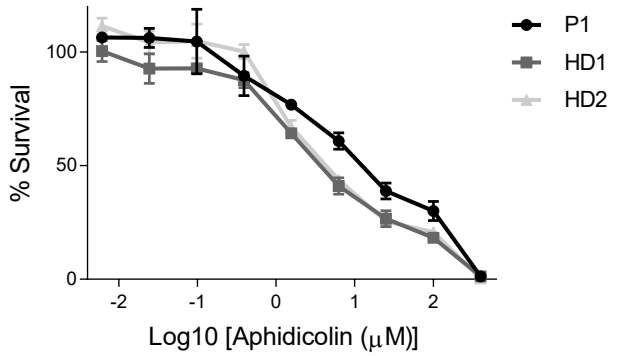
Supplementary Figure 3

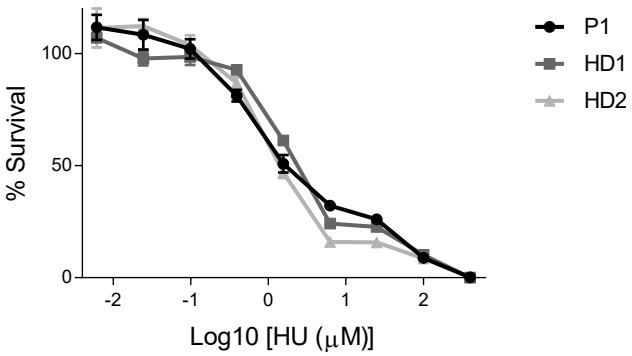


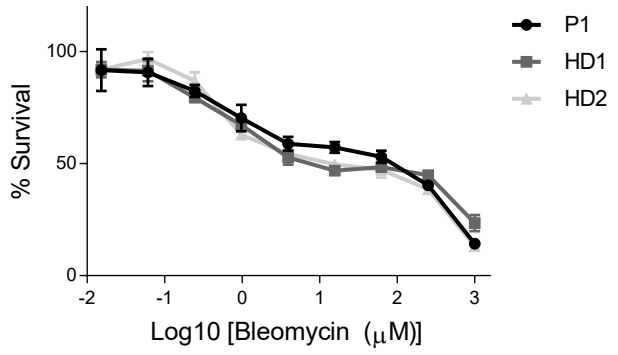


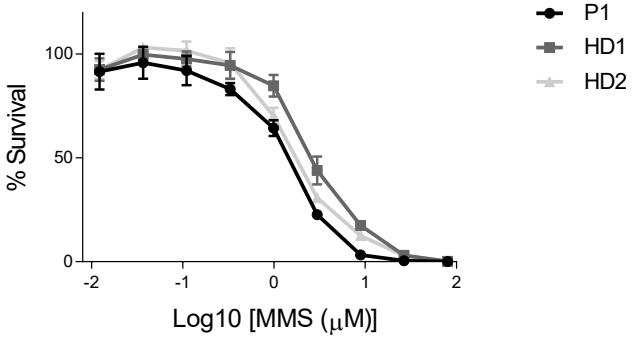


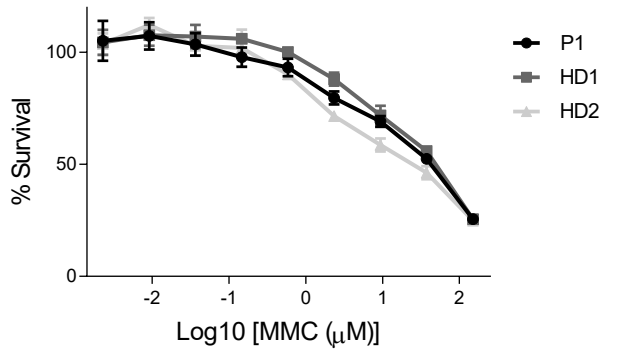
Supplementary Figure 4

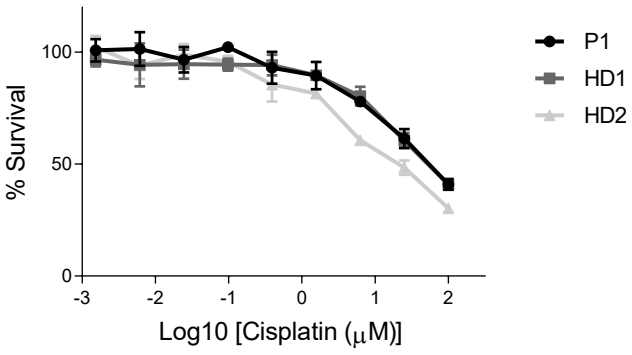


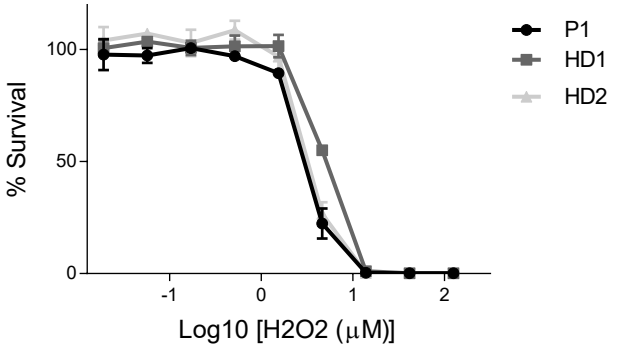




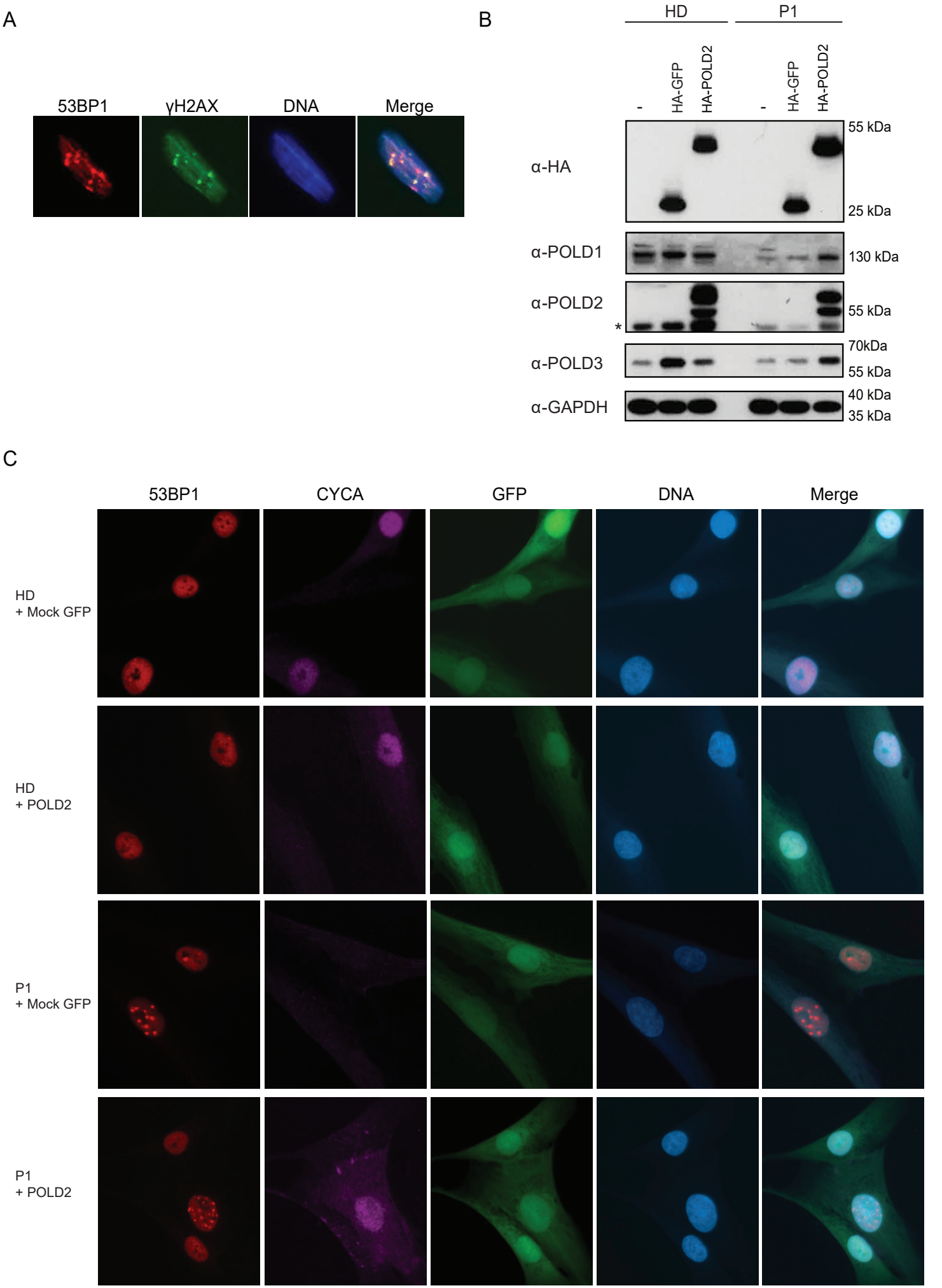








Supplementary Figure 5



Supplementary Figure legends

Figure S1. T-cell immunophenotyping and qPCR. (A) T cell phenotyping showing the relative proportions of naïve cells ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$) and exhausted ($CD45RA^+CCR7^-$) cells within the $CD3^+CD8^+$ T cell population in the lymphocyte gate. **(B)** Histograms showing the expression of CD95 in naïve cells ($CD45RA^+CCR7^+$). (**C**) B cell phenotyping showing the relative proportions of naïve B cells (IgD+CD27-) and class- switched B cells (IgD-CD27+) cells within the CD19+ population in the lymphocyte gate (**D**) Percentage of mutated nucleotides within RGYW/WRCY motifs and percentage of transition mutations at GC sites in the IgA and IgG locus of P1 and normal donor (ND), describing the hotspot motifs of Activation Induced Deaminase (AID)-dependent deamination, a hallmark of phase I of SHM. (**E**) Percentage of mutated nucleotides within WA/TW motifs and percentage of transition mutations at AT in the IgA and IgG locus of P1 and ND, describing the hotspot motif for polymerase η dependent mutations. (**F**) Percentage of transition mutations at GC sites in the IgA and IgG locus of P1 and ND. (**G, H**) RT-qPCR analysis of *POLD1* and *POLD2* relative mRNA expression in healthy donor, P1 and P2 PBMC.

Figure S2. Co-immunoprecipitation and recombinant expression. (A) The position of the Gln684His and Ser939Trp patient mutations and the murine Lys606 mutant in the structure of POLD1 (residues 81-984; model on yeast Pol3 PDB ID 3IAY) is shown. **(B)** Multiple sequence alignment of the region containing the amino acids affected by the identified POLD1 missense mutations (all three indicated by a blue frame). **(C)** Multiple sequence alignment of the region containing the identified POLD2 missense mutation (indicated by a blue frame). **(D)** Strep-Tactin pulldown of wildtype and mutant POLD2-S-HA or GFP-S-HA from nuclear lysates of Jurkat cells stably overexpressing wildtype POLD2 or GFP as a control. (**E**) Coomassie staining of recombinant wildtype and mutant polymerase d complexes. CD, catalytically dead. WT, wildtype.

Figure S3. T cell activation and proliferation. (A) T cell proliferation as measured by violet proliferation dye 450 (VPD450) and expression of the activation markers CD25 and CD95 3 days after stimulation with anti-CD3 and anti-CD28. (**B**) T cell phenotyping showing the relative proportions of naïve cells ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$) and exhausted ($CD45RA^+CCR7^-$) cells within the $CD8^+$ population from *in vitro* expanded T cells. (**C**) Immunoblot analysis of ERK phosphorylation in *in vitro* expanded T cells upon stimulation with anti-CD3 and anti-CD28. HSP90 was used as a loading control. Total and phospho ERK were run on two different gels.

Figure S4. Assessment of HD and P1 fibroblast sensitivity to DNA damaging agents. HD and P1 fibroblast were treated for 5 days with various genotoxic agents including aphidicolin, hydroxyurea, bleomycin, methyl methanesulfonate (MMS), mitomycin C, cisplatin and H_2O_2. Cell survival was measured by Cell Titer-Glo. Data shown is representative of 3 independent experiments.

Figure S5. Reconstitution of POLD2 in P1 fibroblasts using retroviral transduction (A) Immunofluorescence staining of 53BP1 foci and gH2AX in P1 fibroblasts **(B)** Immunoblot analysis of polymerase d complex members in healthy donor and P1 fibroblasts transduced with a control retroviral vector or a retroviral vector containing S-HA-tagged POLD2. Asterisk (*) marks the endogenous POLD2 (**C**) Immunofluorescence staining of 53BP1 foci, cyclin A

and GFP in healthy donor and P1 fibroblasts transduced with control or S-HA-tagged POLD2 containing retrovirus.

Supplementary Table 1. Clinical and Immunological features of the patients.

Patients (Age)	P1	Age-adjusted reference values	P2	Age-adjusted reference values
DEMOGRAPHICS				
Age, yrs	17		24	
Gender	Male		Male	
Consanguinity	Yes		No	
CLINICAL				
Respiratory infections	Sinusitis, otitis, pneumonia		Sinusitis, otitis, pneumonia, bronchitis	
Viral infections	Molluscum contagiosum		Skin warts (suspected topic HPV infection)	
Other infections	Skin abscesses		No	
Neurological findings	Mental retardation, severe intellectual disability, attention deficit, hyperactivity		Microcephaly, low IQ (70), hearing loss	
Autoimmunity	No		No	
Lymphoproliferation	No		No	
Pulmonary complication	Bronchiectasis		Bronchiectasis	
Allergy	No		No	
IMMUNOLOGICAL PARAMETERS				
Age at analysis (yrs) (prior to Ig replacement)	14		19	
IgG (g/l)	4.3	7.7-15.1	10.67	8.0-18.0
IgM (g/l)	0.42	0.70-1.5	1.05	0.6-2.5
IgA (g/l)	0.18	1.08-3.25	1.17	0.9-4.5
$CD3^+$ cells/µl	1014	1033-3325	640	1024-2793
$CD3^+CD4^+$ cells/µl	75	504-1776	50	621-1631
$CD3^+CD8^+$ cells/µl	820	381-1312	510	269-1255
$CD4^+CD45RA^+$ (%)	12.5	35.2-64.4	4	14.4-87.3
$CD4^+CD45RO^+$ (%)	86.5	30.2-71.0	9	33.8-92.4
$CD8^+CD45RA^+$ (%)	45.7	32.6-72.1	18	25.4-92.7
$CD8^+CD45RO^-$ (%)	59.5	17.8-70.3	52	13.4-98.8
$CD4^+CD45RA^+CD31^+$ (%)	12	31-81	7	7-100
$CD19^+$ cells/µl	65	94-792	50	89-540
$CD19^+CD27^-IgD^+$ (%)	49.6	45.7-92.1	22.6	36.1-85.2
$CD19^+CD27^+IgD^+$ (%)	12	4.7-28.3	9.5	7.9-38.2
$CD19^+CD27^+IgD^-$ (%)	20	6.1-35.2	59.7	8.2-44.1
$CD16^+CD56^+$ cells/µl	60	85-680	50	100-640

T cell, $CD3^+$; helper T cell, $CD3^+CD4^+$; cytotoxic T cell, $CD3^+CD8^+$, naive helper T cells, $CD4^+CD45RA^+$; naïve cytotoxic T cells, $CD8^+CD45RA^+$; memory helper T cell, $CD4^+CD45RO^+$; memory cytotoxic T cell, $CD8^+CD45RO^+$; recent thymic emigrants, $CD4^+CD45RA^+CD31^+$; B cell, $CD19^+$; naive B cells, $CD19^+CD27^-IgD^+$; non-switched memory B cells, $CD19^+CD27^+IgD^+$; class-switched memory B cells, $CD19^+CD27^+IgD^-$; natural killer cells, $CD16^+CD56^+$.